CHEMICAL MANUFACTURERS ASSOCIATION

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May 4, 2000

Dr. Jack Moore
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1800 Diagonal Road
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Ethylene Glycol

Re: National Toxicology Program: Center for the Evaluation of Risks to Human Reproduction -- Request for Comments on Candidate Chemicals, 65 Fed. Reg. 14997 (Mar. 20, 2000)

Dear Dr. Moore:

The Ethylene Glycol Panel (EG Panel) [1] of the Chemical Manufacturers Association and the European Chemical Industry Council Ethylene Oxide and Derivatives Producers Association, Toxicology Group (CEFIC Tox Group)[2] submit this letter in response to the inclusion of ethylene glycol on the list of candidate chemicals being recommended for further consideration by the National Toxicology Program (NTP) Center for the Evaluation of Risks to Human Reproduction (CERHR). The EG Panel and the CEFIC Tox Group acknowledge that ethylene glycol may be an appropriate chemical for future consideration by the CERHR, but would strongly encourage the CERHR to defer any actions until the ongoing research programs sponsored by these two groups are completed. The research programs focus on the differences in developmental effects seen between species following oral exposure to ethylene glycol, on the mechanism(s) of action related to these effects and on a comparison of the mechanism(s) with other exposure routes. We believe it would be premature for CERHR to initiate a detailed evaluation of ethylene glycol until these very important data are available for consideration in the process.

OVERVIEW

As stated in the March 20, 2000, *Federal Register* notice, the purpose of the CERHR is "to provide timely and unbiased, scientifically sound evaluations of human and experimental evidence for adverse effects on reproduction, including development, caused by agents to which humans may be exposed." Among the stated goals is providing the general public with information about the strength of scientific evidence that a given exposure will pose a hazard of reproduction or developmental toxicity. The Panel and the CEFIC Tox Group contend that an assessment by CERHR on ethylene glycol in 2000 would not be "timely," and would not provide sufficient information about reproductive and developmental risks associated with specific exposure levels, as there are important data presently being developed that will not be available for assessment purposes until 2001, but that are expected to prove critical in thoroughly understanding the mechanism of ethylene glycol effects. As stated generally above, the objective of the EG

1 Member companies of the CMA EG Panel include Shell Chemical Company, The Dow Chemical Company, Union Carbide Corporation, Eastman Chemical Company, Huntsman Corporation, BASF Corporation, and Equistar Chemicals, LP.

2 Member companies of the CEFIC Ethylene Oxide and Derivatives Producers Association, Toxicology Group include Akzo Nobel, BASF, BP Amoco Chemicals, Clariant, Condea Chemie, Dow Chemical, EC Erdölchemie, Enichem, Ineos, La Seda de Barcelona, Polski Koncern Naftowy, Shell Chemicals, and Union Carbide Europe.

Panel and CEFIC Tox Group's research programs is to better understand the mechanism(s) related to manifestation of developmental toxicity effects following ethylene glycol exposure in different species, how the metabolism of ethylene glycol is altered with increasing dose and rate of administration, and the role that key metabolites play in causing toxic effects. Clearly, understanding the mechanism(s) of how effects may or may not occur in certain species under certain circumstances would be essential in achieving CERHR's goal of determining whether hazards are posed to humans by this chemical.

SUMMARY OF RELEVANT EXISTING RESEARCH PROGRAMS AND REVIEWS THAT PROVIDE INFORMATION CONCERNING PHARMACOKINETICS AND METABOLISM

It is well known that large oral bolus doses of ethylene glycol in some laboratory animals lead to systemic and developmental toxicity. Based on the data developed thus far, it appears that developmental toxicity effects seen in laboratory animals are due to glycolic acid, and may be exacerbated by metabolic acidosis. Since 1994, the EG Panel and the CEFIC Tox Group have sponsored the following reviews and research programs to further explore the hypothesis that glycolic acid is the primary toxicant associated with developmental effects and to better understand the circumstances in which glycolic acid may be produced and the effects it may cause:

"An Integrated Perspective on the Developmental Toxicity of Ethylene Glycol"
(Carney, Reproductive Toxicology, Vol. 8, No. 2, pp. 99-113. 1994)
This article provides an excellent review of the available ethylene glycol data, considers a mechanistic model for toxicity, and adds perspective for addressing concerns with human risks.

"Identification of Proximate Toxicant for Ethylene Glycol Developmental Toxicity Using Rat Whole Embryo Culture"
(Carney, et al., Teratology, Vol. 53, pp. 38-46. 1996)
This study investigated the effects of ethylene glycol and its metabolite, glycolic acid, in rat conceptuses. The results from this study suggest that glycolic acid, not parent ethylene glycol, is the active toxicant for ethylene glycol-included developmental toxicity.

"In-Vitro Skin Penetration of Ethylene Glycol Using Excised Skin from Mice and Humans"
(Sun, et al., Journal of Toxicology - Cutaneous and Ocular Toxicology,
Vol. 14, No. 4, pp. 273-286. 1995)
This study evaluated ethylene glycol for its skin penetration characteristics using *in-vitro* techniques. Full-thickness skin preparations from female CD-1® mice and female human abdominal skin were used. The results indicate that the potential toxicity resulting from cutaneous exposure to ethylene glycol would be significantly less for humans than that predicted by dermal studies using mice.

"Ethylene Glycol: Comparative Pharmacokinetic and Metabolism Probe in Pregnant Rabbits and Rats" (Carney, et al., R&D Report, The Dow Chemical Company, April 22, 1998)
This study was conducted in two phases: (1) a maternal blood time course study examining levels of parent ethylene glycol, glycolic acid and oxalic acid, and acid-base status following gavage administration of ethylene glycol to three auricular (ear) artery-cannulated, gestation day (gd) 9 rabbits; and (2) a probe study to determine if there are any apparent differences between rabbits and rats with respect to levels of ethylene glycol, glycolic acid and oxalic acid in maternal blood and extraembryonic fluids (EEF). Rabbits achieve peak parent ethylene glycol levels one hour after dosing (similar to rodents) and exhibit a broad blood glycolic acid time concentration curve with maximum glycolic acid levels occurring during the 4–12 hour post-dosing interval with no sharp peak. No evidence of metabolic acidosis was observed in these rabbits. Analysis of ethylene glycol and metabolites in part 2 revealed some differences: (1) levels of glycolic acid in both maternal blood and EEF are much lower in rabbits than in rats; and (2) rat embryos exhibit a higher concentration of glycolic acid in the EEF than in maternal blood (apparent "ion trapping"),

whereas glycolic acid concentrations within rabbit EEF tended to be lower than those of maternal blood.

"Dose-Dependent Non-Linear Pharmacokinetics of Ethylene Glycol Metabolites in Pregnant (GD 10) and Non-Pregnant Sprague-Dawley Rats Following Oral Administration of Ethylene Glycol" (Pottenger, et al., manuscript in preparation)
This study described the kinetics of orally administered ethylene glycol and its major metabolites, glycolic acid and oxalic acid, in pregnant (gestation day 10 at dosing, gd10) rats across several dose levels, and between pregnant and non-pregnant (NP) rats. No differences were identified between pregnant (gd 10-11) and non-pregnant female rats for the pharmacokinetic and metabolism parameters investigated. Glycolic acid in blood showed an apparent dose-linearity at doses up to 150 mg EG/kg, but a disproportionally larger increase in glycolic acid blood levels at doses $\geq$500 mg EG/kg. In addition, both ethylene glycol and glycolic acid exhibited dose-dependent urinary elimination at doses of $\geq$500 mg EG/kg, apparently not related to any saturation of renal elimination, but probably due to saturation of metabolic conversion of ethylene glycol to glycolic acid, and of glycolic acid to downstream metabolites. The shift to non-linear kinetics corresponded with the developmental toxicity profile of ethylene glycol in rats (NOEL=500 mg EG/kg; LOEL=1000 mg EG/kg), providing additional evidence for the role of glycolic acid in ethylene glycol developmental toxicity. The peak maternal blood concentration of glycolic acid associated with the LOEL for developmental toxicity in the rat was quite high (363 ug/g or 4.8 mM blood).

"Development Of A PBPK Model For Ethylene Glycol And Its Metabolite, Glycolic Acid" (Corley, et al., presented at the 39th Annual Meeting of The Society of Toxicology, March 19-23, 2000)
A physiologically based pharmacokinetic (PBPK) model was developed to describe the disposition of ethylene glycol and glycolic acid in female rats, including during pregnancy. Metabolic rate constants for ethylene glycol and glycolic acid were estimated and partition coefficients for ethylene glycol and glycolic acid were determined. The PBPK model included inhalation, oral, dermal, intravenous and subcutaneous routes of administration. Metabolism of ethylene glycol and glycolic acid were described in the liver with elimination via the kidneys. Several rat metabolism studies were simulated. Pregnancy had no effect on maternal ethylene glycol and glycolic acid kinetics over a broad dose range. Simulations were consistent with studies indicating that metabolism of ethylene glycol to glycolic acid was essentially first-order (linear) up to 2500 mg/kg/day while the metabolism of glycolic acid saturated between 200 and 1000 mg/kg/day. This resulted in non-linear increases in blood glycolic acid concentrations, which correlate with the toxicity of ethylene glycol.

"*Comparison of metabolism of ethylene glycol in vitro in liver of rat, rabbit and man.*" (Booth, et al., to be presented at Toxicology Forum July 2000)
This study investigated the use of viable tissue slices prepared from livers of female rats, rabbits and humans in dynamic organ culture in vitro, thus maintaining coupled phase I (oxidation) and phase II (conjugation) xenobiotic metabolic activity. Qualitative differences in the metabolic profiles and quantitative differences in formation of glycolic acid between the species were found. There was 3-25 (av 10) fold less glycolic acid produced by rabbit liver slices than rats. Using liver tissue from humans, no glycolic acid was detected. Concentrations of glycolic acid after exposure to ethylene glycol are thus expected to be lower in humans than rats or rabbits. Assuming that glycolic acid is the developmental toxicant in humans as well as rodents, the study demonstrates that MEG is unlikely to present a hazard to man following normal conditions of use and exposure. Comparative studies on the metabolism of glycolic acid with these liver slices clearly demonstrated metabolism to glyoxylic acid with human liver being the most effective. Overall these results caution against the extrapolation of the developmental effects seen in rodents following high bolus doses to humans and hence to classification of MEG as a reproductive toxicant.

SUMMARY OF ONGOING PHARMACOKINETIC AND METABOLISM RESEARCH SPONSORED BY THE EG PANEL AND THE CEFIC TOX GROUP

The focus of the ongoing research program of the EG Panel and the CEFIC Tox Group is to further define the kinetics of ethylene glycol and its metabolites, and includes the following studies:

"Toxicokinetics of Ethylene Glycol and its Metabolites Glycolic Acid and Oxalic Acid in Rats and Humans"(Filser, GSF-Forschungszentrum fur Umwelt and Gesundheit GmbH Institut für Toxikologie)
Ethylene glycol is teratogenic and embryotoxic to rats and mice following oral administration of 750 and 1250 mg/kg/day. However, in rabbits, no observed adverse effect was seen at the highest dose tested (2000 mg/kg/day). According to present knowledge, glycolic acid is the presumed developmental toxicant. Only if the human body burden of glycolic acid is known following human exposure to occupationally relevant concentrations of ethylene glycol can a threshold be determined at which glycolic acid concentrations are too low to result in developmental effects in comparison with data from rats and rabbits. Concentration-time courses of ethylene glycol and glycolic acid in humans and rats following 4-hour exposure to 10 ppm (approximately half the current German Occupational Exposure limit for ethylene glycol) will be determined. Furthermore skin uptake of ethylene glycol will be determined in humans and a data based compartmental model will be used to calculate metabolic parameters and blood concentrations for use in PBPK models of ethylene glycol metabolism.

"Comparison of In Vitro Metabolism of Ethylene Glycol in Rat and Human Liver S-9" (Bartels, The Dow Chemical Company, Toxicology and Environmental and Research Consulting)
The initial phase of the study will be to further examine the metabolic fate of ethylene glycol in vitro, in the presence of rat and human liver cytosol. The major metabolites arising from these incubations will be characterized via 13C-NMR and mass spectrometry. Liver cytosol will be used as the media in these experiments, as the major metabolic routes identified for ethylene glycol are due to enzymes present in the soluble fraction of the liver. In addition, the in vitro kinetics of ethylene glycol and glycolic acid metabolism may be determined in rat and human liver cytosol. These data will be used to aid in the development of a pharmacokinetic model of ethylene glycol in mammalian species.

"Developmental Pharmacokinetics of Slow and Fast Dose-Rate Exposures - Part I" (Carney, The Dow Chemical Company, Health and Environmental Research Laboratory)
This study will generate metabolic data for slow dose-rate exposures to ethylene glycol using surgically implanted slow continuous infusion pumps in rats on gestation day 6. The pumps will be filled with ethylene glycol solutions targeted to deliver approximately 1000 or 2000 mg/kg/day of ethylene glycol. Maternal blood, extraembryonic fluids, and embryo tissues will be collected and analyzed for ethylene glycol and glycolic acid.

"Developmental Pharmacokinetics of Slow and Fast Dose-Rate Exposures - Part II" (Corley, Battelle, Pacific Northwest Division)
This study will be done in conjunction with the study listed above. This study will compare levels of parent ethylene glycol and its metabolite, glycolic acid, in rat maternal blood, extraembryonic fluids, and embryos at various times following a large (saturating) bolus or smaller (non-saturating) bolus dose of ethylene glycol. These data will be compared to those data developed in the slow dose-rate (pump) ethylene glycol exposures (see above).

The data generated in the studies listed above will be essential to better understand the impact of dose and dose-rate associated with toxicity effects related to ethylene glycol exposure. The EG Panel and the CEFIC Tox Group contend that an evaluation of ethylene glycol at this time, without including the data currently being generated, would be incomplete. Thus, we request that ethylene glycol be deferred from consideration as a candidate chemical in 2000.

Please note that the EG Panel and CEFIC Tox Group will be sponsoring a session on ethylene glycol metabolism at the Tox Forum in July 2000. Included in that session will be a panel discussion on the overall strength of the metabolism data and on recommendations on future research considerations. We would encourage CERHR to provide a representative to this panel.

Thank you in advance for your attention to this matter. Please direct any questions concerning the comments to Kathleen Roberts, Manager of the Ethylene Glycol Panel. Ms. Roberts can be reached at 703-741-5613 (telephone), 703-741-6091 (telefax) or Kathleen_Roberts@cmahq.com (e-mail).

Sincerely Yours,

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